Potentially Actionable Suspect Sample (PASS) System – Edited comments from Stakeholders and APHIS' Responses (April 2006):

1. The system used by USDA and its cooperating labs for EIA (Equine Infectious Anemia) seems like an excellent model for *P. ramorum*. They use a three-tiered system; a screening lab, a referral lab, and a "reference" lab. The screening lab has been approved to perform a specific test for the disease. If they get a positive, they must repeat it. If it is positive a second time with that same test, the sample is sent on to the referral lab. The referral lab repeats the same test on the sample and, if positive, performs a second USDA-approved test on the sample. The second test is replicated. If both replications of the second test are positive, the referral lab has the authority to declare an officially confirmed positive. If there is any discrepancy (e.g., one replication comes back negative), the sample must be sent on to the reference lab for further testing (usually with a third and/or fourth test) and the final call on the diagnosis. Likewise, the referral lab can call a negative if, for example, the second test is negative in both replications.

APHIS comments on above: We are proposing a similar two-tiered system. In our system, a presumptive positive (assuming it is a PASS sample) is sent to the 'reference laboratory', which in this case would be an APHIS PPQ lab. If the external approved lab obtains a positive, they do have the option of repeating the test(s) and for certain types of samples must resample and retest. APHIS strongly recommends that all presumptive positives be re-tested.

2. The PASS system as written looks no different than the current system which is too slow when dealing with large sample numbers. The developed system for Equine Infectious Anemia would allow states to respond more quickly to detections/interceptions of *P. ramorum* and would alleviate much of the pressure put on the USDA labs by large sample numbers. It would also return some confidence in the entire process to the nurseries and others who are currently being impacted by the existing and this new system.

APHIS comments on above: It is true that in part of 2005, there were delays in obtaining laboratory results from APHIS due to the large number of samples in the cue. Since something on the order of 90% of those samples was negative, had this new policy been in place last year and had there been Provisionally Approved labs to perform the tests, these delays would likely not have occurred. Note that the APHIS lab in Beltsville is caught up and has been for some time. Occasionally, a sample is tested that requires considerable additional work (e.g., other tests, sequencing, etc.,), and these can take some time. However, this occurs in less than 1% of the samples.

The proposed system is intended to accomplish the same as the EIA system. Our intent is to only have APHIS laboratories test those samples that require Federal confirmatory testing. Again, if this policy had been in place last year, something on the order of 90% of the samples would have been eliminated from Federal testing. It is also worth noting that APHIS is in the process of establishing a dedicated molecular diagnostics laboratory

whose primary purpose will be to perform 'routine' diagnostic tests.

3. Any positive or presumptive positive from any site or from nature should be considered a PASS sample, not just those specified in the Sample Routing Section.

APHIS comments on above: We agree, in principle. The intent of the policy is that any sample that is from certain natural/environmental finds should receive Federal confirmatory testing, assuming that the sample meets the requirements of a PASS sample.

4. Clarification is needed on what state or university laboratories that have not yet been certified by APHIS should do with *P. ramorum* samples in various programs settings. If a trace forward plant at a trace forward site in another State is determined to be positive by a Provisionally Approved lab in that State, that sample is a PASS sample. If that sample is determined to be positive by a laboratory in the recipient State that is not Provisionally Approved, the sample results, positive or negative must be sent to Beltsville, or the DNA extract if only ELISA was done on the sample(s) taken from the trace forward plant(s).

Both the PASS policies and Emergency Federal order suggest that only APHIS and certain laboratories can process samples, but this new PASS policy is trying to reduce number of samples submitted to APHIS reference laboratories.

APHIS comments on above: This seems to be a restatement of existing policy. If a laboratory which is not a Provisionally Approved lab obtains a positive ELISA result, then they are to extract DNA and have that DNA tested at Beltsville or by a Provisionally Approved lab.

5. In the introduction it is stated that the Federal Emergency Order effective January 10, 2005, specifies which samples are to be submitted to APHIS laboratories for confirmation. This is not true. In the Federal Order, Section V only states that "Samples collected at nurseries and regulated areas must be analyzed using a methodology approved by APHIS at a laboratory approved by APHIS." Our state lab has already complied with their requirements (inspection, proficiency panel, equipment, personnel qualifications, etc.) so far and it is a "Provisionally Approved Lab".

APHIS comments on above: This is correct, and appropriate changes are reflected in this document version. There is a Provisionally Approved laboratory in this state. However, note that this policy clarifies what laboratories are "approved by APHIS" for what type of samples and even Provisionally Approved Laboratories must forward certain samples for further analysis.

6. We have been sending the initial *P. ramorum* positives to Beltsville. This PASS protocol does not accomplish anything more, and definitely does not reduce the time needed to determine if *P. ramorum* is present as it claims**.** On the contrary, it requires all environmental samples (initial and subsequent finds) to go to Beltsville, which will complicate the situation not only for the researchers, but more so for us – due to the many samples we get from known infested counties.

APHIS comments on above: Samples from the infested counties would not be PASS samples, unless they represent a new host or an area not previously established as infested, thus requiring Federal action. So an environmental sample from a county not previously known to be infested would be considered a PASS sample. Subsequent samples from that hypothetical county would not necessarily be PASS samples. Therefore, the total number of samples requiring Federal confirmatory sampling would be quite low.

7. This new PASS still doesn't solve our problem of having our nurseries on regulatory hold, while APHIS takes additional time to "confirm" results that we have already completed and are ready to take legal regulatory action on. If this piece of the agreement is in the final version, we're going to have to come up with some type of policy in which we (and APHIS) allow an infested nursery to actually begin cleanup and start the clock on the compliance process based on our test results, and not have to wait for "official" APHIS results. Otherwise, impacted nurseries are likely to miss their market windows. Our (major) "shipper" state is in an entirely different position from the other "recipient" states or from other *P. ramorum* infested states.

APHIS comments on above: In principle, we agree. The State may initiate action at the time they observe the first presumptive positive, and the “CNP (Confirmed Nursery Protocol) clock” should start at that time. The CNP 90 day quarantine should commence only when all samples have been taken. From these samples the quarantine blocks will be defined. The 90 day CNP quarantine should not commence prior to completion of delimitation survey, that is, once all samples are taken. However, until a Federal confirmatory test is conducted, the state would have to accept responsibility for the test results and any action taken under State authority.

8. In addition, it appears that positive results based on samples from which a laboratory successfully cultures *P. ramorum* will no longer be recognized until the cultures are also confirmed by APHIS. Prior to this, APHIS recognized positives based on live cultures without PCR tests. Now APHIS wants to do away with that without providing rational or having a discussion over the issue.

APHIS comments on above: The National Identification Service would have to issue identification authority which would apply only to cultures not falling under PASS. NIS has been confirming identification of cultures throughout the life of this program. In the early stages of the program, APHIS was conducting PCR tests on positive cultures, but

that has since been determined to be redundant. The cultures can be confirmed very shortly after receipt by NIS, if they are sporulating, and thus should not add substantial time to the confirmation process. Again, the number of cultures that would require Federal confirmatory identification should be rather low.

9. Closing comments: Please keep in mind that developing this policy for *P. ramorum* is arguably the most complex of situations. The overriding principle is that any diagnosis from which Federal action may ensue or where Federal funds and/or action are involved must be subject to Federal confirmatory testing. This is intended to protect all parties involved. Clearly, in the relatively rare instances where the state lab and the Federal lab disagree on assay results, more testing and/or sampling will be required.

We have, in essence, established a PASS sample policy for two other high consequence plant diseases: Asian soybean rust and huanglongbing (HLB or Citrus greening). In the former case, a PASS sample is the first sample from a previously uninfested state or a new host to the US, and in the case of HLB, it is simply any presumptive positive from a county in Florida not previously identified as positive, or a new host. In the case of soybean rust, this policy has been officially communicated. In the case of HLB, the policy is an agreement between the State and the Federal government. In any case, both systems are considerably less complex than is the case with *P. ramorum*. In the future, we hope to develop relatively generic PASS sample policies that would allow us to relatively easily modify for specific pathosystems.